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# UV, stress and aging

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**Key words:** UVB, fibroblasts, keratinocytes, senescence

**Abbreviations:** CPD, cumulative population doubling; HDF, human diploid fibroblast; NHEK, normal human epidermal keratinocyte; ROS, reactive oxygen species; SA- $\beta$ gal, senescence-associated  $\beta$ -galactosidase; UV, ultraviolet

Skin is a model of choice in studies on aging. Indeed, skin aging can be modulated by internal and external factors, reflecting its complexity. Two types of skin aging have been identified: intrinsic, mainly genetically determined and extrinsic—also called “photo-aging”—resulting on the impact of environmental stress and more precisely of UV rays. Simplified in vitro models, based on cellular senescence, have been developed to study the relationship between UV and aging. These models vary on the cell type (fibroblasts or keratinocytes, normal or immortalized) and the type of UV used (UVA or UVB).

## Introduction

Skin is a model of choice in studies on aging. Indeed, aging of the skin can be modulated by internal and external factors, reflecting its complexity. Two different types of skin aging are identified: intrinsic aging, mainly genetically determined and similar to aging of other organs, and extrinsic aging, resulting on the impact of environmental stress and more precisely of UV rays (for a review see refs. 1 and 2). If these two types of skin aging present differences at the morphological and at the histological levels,<sup>1,2</sup> they share molecular similarities (for a review see ref. 3) as the induction of matrix metalloproteinases. In order to better understand skin photo-aging, and more precisely, the relation between UV and aging, several simplified in vitro models have been developed, based on in vitro models of cellular senescence.

## Skin Aging

Skin aging can be modulated by external factors. This extrinsic aging is superimposed on intrinsic one, and is also referred as “photo-aging.” Indeed, if several exogenous factors as tobacco smoke, infrared radiation, pollution, malnutrition etc. can interfere with skin aging, the factor having the greatest impact is clearly UV rays. Naturally, intrinsic and extrinsic aging of the

skin are observable in the same individual depending on whether the parts of skin were protected from the sun or not. The face and the backside of the hands are usually the most photoaged affected areas. Clinically, intrinsically aged skin is thin, smooth and presents only light wrinkles.<sup>2,4</sup> Different subtypes can characterize extrinsic aging of the skin. Classically, it is distinguished by a thicker skin (leathery aspect), with deeply marked wrinkles and an irregular pigmentation (age spots).<sup>2,4</sup> Histologically, both types of skin aging are characterized by change in the organization of structural components of the connective tissue. Intrinsically aged skin is marked by a decrease in epidermal and dermal thickness. The interstitial collagen and elastin content are reduced while collagen cross-links fibers content is increased.<sup>2,4</sup> Extrinsically aged skin shows hyperplasia, with an increase of the thickness of the epidermis and dermis. There is a complete perturbation of the structural content (reduced interstitial collagen, increased elastic fibers) associated with damaged fibers leading to a severe disorganization of the connective tissue structure.<sup>2,4</sup> Aging of the individuals appears to be linked to internal factors as genetic predispositions (as shown for longevity<sup>5–7</sup>), hormonal status<sup>8</sup> and to environmental factors. The degree of influence of these genetic and environmental factors has not been clearly described in aging of the skin.<sup>9</sup> However, several studies of cohorts of twins helped highlight the importance of these two factors.<sup>10,11</sup> Despite their differences, evidence shows that intrinsic and extrinsic aging of the skin are probably driven by similar biological, biochemical and molecular mechanisms.<sup>12</sup> Thus, the formation of reactive oxygen species (ROS) and the induction of matrix metalloproteinases are shown to be common factors of both types of skin aging.<sup>3</sup> It is assumed that ROS accumulation detected in intrinsic and extrinsic aging leads to the activation of MAPK (mitogen-activated protein kinases) pathways. ERK (extracellular signal-regulated kinases), JNK (c-Jun N-terminal kinase) and p38<sup>MAPK</sup> once activated induce the activation of AP-1 (activator protein-1) transcription factor. AP-1 induces collagen degradation by promoting the expression of matrix metalloproteinases MMP-1, MMP-3 and MMP-9<sup>12,13</sup> and by preventing the expression of procollagen-1.<sup>14</sup>

## UV and Photo-Aging

UV are essential components of sunlight. In vivo, skin is exposed to UVB and UVA as UVC are stopped by the ozone layer. UVB

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(290–320 nm) and UVA (320–400 nm) are able to cross the epidermis and to reach the dermis.<sup>15</sup> UVB and UVA can interact with endogenous chromophores and photosensitizers resulting in the generation of ROS causing damage to DNA, proteins and lipids. Moreover, UVB can directly interact with DNA and generate dipyrimidine photoproducts such as cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidone photoproducts (for a review see refs. 16 and 17). UVB are therefore considered as the most harmful UV rays. UV radiation activates several signal transduction pathways related to growth, differentiation, senescence and connective tissue degradation<sup>18</sup> by the activation of several cell surface receptors. This includes cytokines or growth factors receptors as the receptors for epidermal growth factor (EGF), tumor necrosis factor (TNF), interleukin-1 (IL-1)<sup>15</sup> and keratinocyte growth factor (KGF).<sup>19</sup> The biological responses to UV can be immediate and transient (inflammation, sunburn cell formation, pruritus) or delayed and chronic (photo-aging, immunosuppression, carcinogenesis). Exposures to UV rays are also used in dermatology to treat many skin diseases including psoriasis, atopic dermatitis, vitiligo, etc.<sup>20</sup>

In most cases, studies on photo-aging require the participation of human volunteers. This implies ethical constraints and limits sample size. In order to investigate in vitro photo-aging, various models have been developed, based on cellular senescence.

### Replicative Senescence and Stress Induced Premature Senescence

In vitro, proliferative somatic cells, as human diploid fibroblasts (HDF), have a limited capacity of cellular divisions. Replicative senescence is defined as an irreversible growth arrest. This was first described by Hayflick in the early 1960s on HDFs<sup>21</sup> and was later extended to most proliferative cell types (for a review see ref. 22). This growth arrest is established and maintained by the p53/p21<sup>WAF-1</sup> and the p16<sup>INK-4A</sup>/pRb pathways.<sup>22</sup> Thirty years later, Harley et al.<sup>23</sup> showed that telomeres shorten during aging of HDFs, and replicative senescence was associated to a “critical” shortening of the telomeres. Cells can remain alive for several months after the onset of replicative senescence<sup>24</sup> and show apoptosis resistance.<sup>25</sup> Several markers can identify senescent cells in vitro and in vivo, however, none is exclusive to the senescent state.<sup>22</sup> Among these biomarkers are: typical enlarged and flattened morphology,<sup>24</sup> senescence-associated  $\beta$ -galactosidase activity (SA- $\beta$ gal),<sup>26</sup> senescence-associated DNA-damage foci (SDFs),<sup>27,28</sup> altered gene expression<sup>29,30</sup> and the common mitochondrial DNA deletion of 4,977 bp.<sup>31</sup> More recently, it was shown that senescent cells secrete growth factors, proteases, chemokines and inflammatory cytokines allowing them to interact with their cellular environment. This was termed senescence-associated secretory phenotype (SASP).<sup>32,33</sup>

Some of these biomarkers are also detected in vivo. For instance, in the skin, some of these biomarkers are also detected during aging: typical senescent morphology,<sup>2</sup> SA- $\beta$ gal activity,<sup>26</sup> p16<sup>INK-4A</sup> overexpression<sup>34</sup> and the “common” mitochondrial DNA deletion.<sup>35</sup>

In vitro, it is possible to induce the appearance of these biomarkers by exposing human proliferative cell types, such as HDFs, endothelial cells, melanocytes, etc. to acute stress at sublethal doses of stressing agents inducing oxidative stress and/or DNA damage. This was defined as “Stress Induced Premature Senescence” (SIPS) and is detectable at 3 d after the stress,<sup>36</sup> long before the cells reach the critical telomere length observed in replicative senescence. Cells in SIPS induced by subcytotoxic concentrations of oxidative agents such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)<sup>37</sup> or *tert*-butylhydroperoxide (*t*-BHP)<sup>31</sup> remain alive for months and display several features of replicative senescence. These features include senescent morphology,<sup>24,31</sup> SA- $\beta$ gal activity,<sup>31,38</sup> common mitochondrial deletion<sup>31</sup> and altered gene and protein expression.<sup>39,40</sup>

In order to study the long-term effect of UV on skin cell types, several in vitro models were developed. Indeed, if multiple studies dealt with the effects of UV very few investigate the long-term effects of UV. Here we present different models that were developed in order to study long-term effects of subcytotoxic doses of UV. Several models were set up varying on the cell type (fibroblasts or keratinocytes, normal or immortalized) and the type of UV used (UVA or UVB).

### UV-Stress Induced Premature Senescence in Fibroblasts

Fibroblasts constitute the classical cell type for studies on aging. Historically, it was the first strain on which replicative senescence was detected<sup>21</sup> and constitutes since the cell type of reference for studies of aging.<sup>22</sup> In the skin, fibroblasts constitute the main cell type of the dermis and are responsible of the production of the different extracellular matrix (ECM) components.<sup>41</sup>

Models of premature senescence were developed by exposing dermal HDFs to sublethal doses of UVB.<sup>42,43</sup> After ten repeated exposures to sublethal dose of UVB, HDFs display biomarkers of senescence as typical senescent morphology, SA- $\beta$ gal activity, altered gene expression and the “common” mitochondrial DNA deletion.<sup>42,43</sup> As the UV dose used must not induce mortality or apoptosis, caspase-3 activity and PARP cleavage were checked as negative in these conditions. The decreased proliferative potential observed after these UVB stresses are correlated with overexpression of p53, p21<sup>WAF-1</sup> and p16<sup>INK-4A</sup>, involved in the growth arrest in replicative senescence. Concerning gene expression change observed after these repeated UVB exposures, the relative steady-state mRNA level of c-jun, c-fos, MMP-1 and MMP-2 was found to be increased. C-fos and c-jun are known to be components of the c-jun:c-fos AP-1 transcription factor. A transient increase of extracellular release of H<sub>2</sub>O<sub>2</sub> was detected after repeated UVB exposures.<sup>44</sup> This model has been accepted as an in vitro simplified model of dermal aging. It was used to identify potential protective effect of marine algal components.<sup>45</sup> Moreover, it was shown that telomerase activity did not prevent premature senescence induced by UVB<sup>46</sup> as telomerase-immortalized human foreskin fibroblasts (hTERT-BJ1) developed biomarkers of senescence after UVB exposures.

At the mechanistic level, the pathways inducing senescence are still unknown. In SIPS, the role of Transforming Growth

Factor- $\beta$ 1 (TGF- $\beta$ 1) in the appearance of some of the biomarkers of senescence has been described. TGF- $\beta$ 1 is a multifunctional cytokine involved in many cellular functions like cellular division, differentiation and connective tissue synthesis.<sup>47</sup> TGF- $\beta$ 1 is secreted in a latent form (LTGF- $\beta$ ), which consists of TGF- $\beta$ 1 noncovalently associated with its N-terminal propeptide called latency associated peptide (LAP) (for a review see ref. 48). The abundance of TGF- $\beta$ 1 mRNA is increased in premature senescence induced by H<sub>2</sub>O<sub>2</sub>,<sup>38</sup> *t*-BHP,<sup>49</sup> ethanol<sup>49</sup> and UVB.<sup>43</sup> By using neutralizing antibodies, it was shown that TGF- $\beta$ 1 is responsible for the appearance of several biomarkers of senescence induced by these stresses.<sup>38,43,49</sup> In premature senescence induced by H<sub>2</sub>O<sub>2</sub>, it was shown that H<sub>2</sub>O<sub>2</sub> induces a first phase of activation of p38<sup>MAPK</sup> (ref. 50). This activation triggers an overexpression of TGF- $\beta$ 1, which starts a positive feedback loop allowing sustained activation of p38<sup>MAPK</sup>. P38<sup>MAPK</sup> phosphorylates and activates the transcription factor ATF-2 that interacts with hypophosphorylated pRb. This complex induces the appearance of features of replicative senescence. This regulatory loop probably also triggers UVB-induced premature senescence with concomitant activation of p53. Indeed, UVB-induced premature senescence is associated with a transient increase of p53 protein abundance and DNA-binding activity. Silencing p53 expression with small interfering RNA (siRNA) affected the basal level of SA- $\beta$ gal and proliferative potential, as the expression of genes differentially expressed after repeated exposures to UVB.<sup>51</sup>

Concerning long-term effects of UVA exposures on HDFs, Herrmann et al. showed that treatment of HDFs with 8-methoxypsoralen and subsequent UVA irradiation resulted in a long-term growth arrest, alterations in cell morphology (post mitotic phenotypes) and increased expression of SA- $\beta$ gal.<sup>52</sup> Combined treatment of psoralen and UVA, also known as pUVA therapy, is widely used in the treatment of different skin disorders as psoriasis. Psoralens act as photosensitizers via the generation of ROS.<sup>53</sup> The long-term growth arrest of pUVA-treated HDFs is associated to increased protein levels of p53, p21<sup>WAF-1</sup> and p16<sup>INK-4A</sup> (ref. 54) and change of expression of genes involved in growth arrest, stress response, modification of the extracellular matrix and senescence.<sup>55</sup> However, pUVA-induced growth arrest, senescent morphology, SA- $\beta$ gal increased activity and MMP-1 overexpression are fully reversible at days 100 to 130 post pUVA treatment. This suggests that pUVA-induced changes do not fully reflect replicative senescence in HDFs but rather represent a long-term transient phenocopy of senescence<sup>56</sup> and was therefore presented as a SIPS model.<sup>57</sup>

For UVA irradiation alone, Berneburg et al. showed that the common mitochondrial deletion was detectable in HDFs exposed to 36 repeated sublethal doses of UVA radiation.<sup>58</sup> This deletion was found to be mediated by singlet oxygen.

### UVB-Stress Induced Premature Senescence or Alternative Differentiation in Keratinocytes

Normal human epidermal keratinocytes (NHEK) constitute the main cell type of the epidermis and are the first line of skin's defense against environmental stresses. They proliferate

in the basal layer before moving upwards to the suprabasal layers through a complex differentiation program that culminates in fully differentiated dead cells in the cornified superficial layer, maintaining a strong impermeable barrier.<sup>59</sup> If it has been detected that keratinocytes show characteristics of replicative senescence *in vivo*<sup>26</sup> very little was devoted to study the aging of keratinocytes *in vitro*. Moreover, very few are known on the long-term effect of subcytotoxic UV exposures on NHEKs. NHEKs progressively show proliferation arrest and reach a senescence plateau after about 15–25 population doublings (according to the donor) in culture.<sup>60</sup> This plateau lasted only a few days to 2–3 weeks and is followed by a massive detachment of almost all cells. Some remaining cells with partially transformed and tumorigenic traits will then spontaneously emerge from senescent cultures, linked to the accumulation of ROS during senescence.<sup>61</sup> Senescent keratinocytes display morphological changes as increase of cytoplasm size and of perinuclear organelle contents.<sup>62</sup> They also display increased SA- $\beta$ gal activity<sup>26,60</sup> and p16<sup>INK-4A</sup> expression.<sup>62</sup>

Lewis et al.<sup>63</sup> demonstrated that low doses of UVB irradiation induce cellular senescence in NHEKs with increased SA- $\beta$ gal activity and increased p21<sup>WAF-1</sup> and p53 protein abundance. Activation of IGF-1R promotes this UVB-premature senescence through increased generation of ROS and by maintaining the expression of p21<sup>WAF-1</sup>.<sup>63</sup>

Telomerase expression alone is not sufficient to immortalize human keratinocytes. Indeed, normal human oral keratinocytes (NHOK) expressing telomerase enter in replicative senescence after several population doublings in culture.<sup>64,65</sup> To be immortalized, keratinocytes should express telomerase and lack p16<sup>INK-4A</sup>. As described earlier, p16<sup>INK-4A</sup> is a strong biomarker of skin aging.<sup>34</sup> Immortalized keratinocytes lacking p16<sup>INK-4A</sup> and expressing telomerase retain other growth controls and keep the ability to differentiate in reconstructed epidermis *in vitro*.<sup>66</sup> Repeated exposures to UVB of immortalized keratinocytes induce an alternative state of differentiation rather than stress-induced premature senescence.<sup>67</sup> This alternative differentiation state is characterized namely by an increased abundance of involucrin, a late marker of differentiation, and cytokeratins (K) K6, K16 and K17, phosphorylation of p38<sup>MAPK</sup> and HSP27, and elevated secretion of active MMP-9, as observed in primary keratinocytes and *in vivo* in the epidermis.<sup>67</sup> Expressions of proteins involved in keratinocyte differentiation and survival were shown to be changed after UVB exposures. Among them, TRIM 29 (TRIPartite Motif Protein 29), a survival factor, is dependent on PKC $\delta$  signaling pathway.<sup>68</sup> These results suggest that p16<sup>INK-4A</sup> is essential for keratinocytes to enter into senescence.

### Conclusions

In conclusion, several models were developed in which human fibroblasts or keratinocytes, exposed to subcytotoxic doses of UV rays, show characteristics of cellular senescence. These models can be used to better understand the relationship between UV stress and aging. Since the skin is a complex organ, consisting of different compartments with connections between them,



it would be interesting to develop in the future more complex models allowing to take into account interactions between the different cell types of the skin. In addition, aging of the skin is modulated by multiple internal (genetic, hormonal) and external (oxidative stress, UV, pollution) factors, which makes it complex to study. However, this complexity makes it an interesting model to use the skin as a picture of the overall aging of the individual.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### References

- Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al. Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 2002; 138:1462-70; PMID:12437452; <http://dx.doi.org/10.1001/archderm.138.11.1462>
- Wlaschek M, Tancheva-Poór I, Naderi L, Ma W, Schneider LA, Razi-Wolf Z, et al. Solar UV irradiation and dermal photoaging. *J Photochem Photobiol B* 2001; 63:41-51; PMID:11684450; [http://dx.doi.org/10.1016/S1011-1344\(01\)00201-9](http://dx.doi.org/10.1016/S1011-1344(01)00201-9)
- Kohl E, Steinbauer J, Landthaler M, Szeimies RM. Skin ageing. *J Eur Acad Dermatol Venereol* 2011; 25:873-84; PMID:21261751; <http://dx.doi.org/10.1111/j.1468-3083.2010.03963.x>
- Ma W, Wlaschek M, Tancheva-Poór I, Schneider LA, Naderi L, Razi-Wolf Z, et al. Chronological ageing and photoaging of the fibroblasts and the dermal connective tissue. *Clin Exp Dermatol* 2001; 26:592-9; PMID:11696063; <http://dx.doi.org/10.1046/j.1365-2230.2001.00905.x>
- Beekman M, Blanché H, Perola M, Hervonen A, Bezrukov V, Sikora E, et al. The GEHA consortium. Genome-wide linkage analysis for human longevity: Genetics of Healthy Ageing Study. *Aging Cell* 2013; PMID:23286790; <http://dx.doi.org/10.1111/acel.12039>
- Kerber RA, O'Brien E, Boucher KM, Smith KR, Cawthon RM. A genome-wide study replicates linkage of 3p22-24 to extreme longevity in humans and identifies possible additional loci. *PLoS One* 2012; 7:e34746; PMID:22506048; <http://dx.doi.org/10.1371/journal.pone.0034746>
- Boyden SE, Kunkel LM. High-density genomewide linkage analysis of exceptional human longevity identifies multiple novel loci. *PLoS One* 2010; 5:e12432; PMID:20824210; <http://dx.doi.org/10.1371/journal.pone.0012432>
- Makrantonaki E, Schönknecht P, Hossini AM, Kaiser E, Katsouli MM, Adjaye J, et al. Skin and brain age together: The role of hormones in the ageing process. *Exp Gerontol* 2010; 45:801-13; PMID:20719245; <http://dx.doi.org/10.1016/j.exger.2010.08.005>
- Gunn DA, Rexbye H, Griffiths CE, Murray PG, Fereday A, Catt SD, et al. Why some women look young for their age. *PLoS One* 2009; 4:e8021; PMID:19956599; <http://dx.doi.org/10.1371/journal.pone.0008021>
- Martires KJ, Fu P, Polster AM, Cooper KD, Baron ED. Factors that affect skin aging: a cohort-based survey on twins. *Arch Dermatol* 2009; 145:1375-9; PMID:20026845; <http://dx.doi.org/10.1001/archdermatol.2009.303>
- Doshi DN, Hanneman KK, Cooper KD. Smoking and skin aging in identical twins. *Arch Dermatol* 2007; 143:1543-6; PMID:18087005; <http://dx.doi.org/10.1001/archderm.143.12.1543>
- Rittié L, Fisher GJ. UV-light-induced signal cascades and skin aging. *Ageing Res Rev* 2002; 1:705-20; PMID:12208239; [http://dx.doi.org/10.1016/S1568-1637\(02\)00024-7](http://dx.doi.org/10.1016/S1568-1637(02)00024-7)
- Fisher GJ, Voorhees JJ. Molecular mechanisms of photoaging and its prevention by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin in vivo. *J Invest Dermatol Symp Proc* 1998; 3:61-8; PMID:9732061
- Chung JH, Kang S, Varani J, Lin J, Fisher GJ, Voorhees JJ. Decreased extracellular-signal-regulated kinase and increased stress-activated MAP kinase activities in aged human skin in vivo. *J Invest Dermatol* 2000; 115:177-82; PMID:10951233; <http://dx.doi.org/10.1046/j.1523-1747.2000.00009.x>
- Rosette C, Karin M. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 1996; 274:1194-7; PMID:8895468; <http://dx.doi.org/10.1126/science.274.5290.1194>
- Hiraku Y, Ito K, Hirakawa K, Kawanishi S. Photosensitized DNA damage and its protection via a novel mechanism. *Photochem Photobiol* 2007; 83:205-12; PMID:16965181; <http://dx.doi.org/10.1562/2006-03-09-IR-840>
- Ichihashi M, Ueda M, Budiyo A, Bito T, Oka M, Fukunaga M, et al. UV-induced skin damage. *Toxicology* 2003; 189:21-39; PMID:12821280; [http://dx.doi.org/10.1016/S0300-483X\(03\)00150-1](http://dx.doi.org/10.1016/S0300-483X(03)00150-1)
- Helenius M, Mäkeläinen L, Salminen A. Attenuation of NF-kappaB signaling response to UVB light during cellular senescence. *Exp Cell Res* 1999; 248:194-202; PMID:10094826; <http://dx.doi.org/10.1006/excr.1999.4393>
- Marchese C, Maresca V, Cardinali G, Belleudi F, Ceccarelli S, Bellocchi M, et al. UVB-induced activation and internalization of keratinocyte growth factor receptor. *Oncogene* 2003; 22:2422-31; PMID:12717419; <http://dx.doi.org/10.1038/sj.onc.1206301>
- Walker D, Jacob H. Phototherapy in the age of biologics. *Semin Cutan Med Surg* 2011; 30:190-8; PMID:22123416; <http://dx.doi.org/10.1016/j.sder.2011.08.004>
- Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961; 25:585-621; PMID:13905658; [http://dx.doi.org/10.1016/0014-4827\(61\)90192-6](http://dx.doi.org/10.1016/0014-4827(61)90192-6)
- Campisi J, d'Adda di Fagnana F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol* 2007; 8:729-40; PMID:17667954; <http://dx.doi.org/10.1038/nrm2233>
- Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990; 345:458-60; PMID:2342578; <http://dx.doi.org/10.1038/345458a0>
- Bayreuther K, Rodemann HP, Hommel R, Dittmann K, Albiez M, Franz PI. Human skin fibroblasts in vitro differentiate along a terminal cell lineage. *Proc Natl Acad Sci U S A* 1988; 85:5112-6; PMID:3393534; <http://dx.doi.org/10.1073/pnas.85.14.5112>
- Hampel B, Malisan F, Niederegger H, Testi R, Jansen-Dürr P. Differential regulation of apoptotic cell death in senescent human cells. *Exp Gerontol* 2004; 39:1713-21; PMID:15582287; <http://dx.doi.org/10.1016/j.exger.2004.05.010>
- Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 1995; 92:9363-7; PMID:7568133; <http://dx.doi.org/10.1073/pnas.92.20.9363>
- d'Adda di Fagnana F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 2003; 426:194-8; PMID:14608368; <http://dx.doi.org/10.1038/nature02118>
- Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Curr Biol* 2003; 13:1549-56; PMID:12956959; [http://dx.doi.org/10.1016/S0960-9822\(03\)00542-6](http://dx.doi.org/10.1016/S0960-9822(03)00542-6)
- Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. *Curr Biol* 1999; 9:939-45; PMID:10508581; [http://dx.doi.org/10.1016/S0960-9822\(99\)80420-5](http://dx.doi.org/10.1016/S0960-9822(99)80420-5)
- Yoon IK, Kim HK, Kim YK, Song IH, Kim W, Kim S, et al. Exploration of replicative senescence-associated genes in human dermal fibroblasts by cDNA microarray technology. *Exp Gerontol* 2004; 39:1369-78; PMID:15489060; <http://dx.doi.org/10.1016/j.exger.2004.07.002>
- Dumont P, Burton M, Chen QM, Gonos ES, Fripiat C, Mazarati JB, et al. Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast. *Free Radic Biol Med* 2000; 28:361-73; PMID:10699747; [http://dx.doi.org/10.1016/S0891-5849\(99\)00249-X](http://dx.doi.org/10.1016/S0891-5849(99)00249-X)
- Coppé JP, Patil CK, Rodier F, Krtolica A, Beauséjour CM, Parrinello S, et al. A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS One* 2010; 5:e9188; PMID:20169192; <http://dx.doi.org/10.1371/journal.pone.0009188>
- Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008; 6:2853-68; PMID:19053174
- Ressler S, Bartkova J, Niederegger H, Bartek J, Scharffetter-Kochanek K, Jansen-Dürr P, et al. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell* 2006; 5:379-89; PMID:16911562; <http://dx.doi.org/10.1111/j.1474-9726.2006.00231.x>
- Berneburg M, Plettenberg H, Medve-König K, Pfahlberg A, Gers-Barlag H, Gefeller O, et al. Induction of the photoaging-associated mitochondrial common deletion in vivo in normal human skin. *J Invest Dermatol* 2004; 122:1277-83; PMID:15140232; <http://dx.doi.org/10.1111/j.0022-202X.2004.22502.x>

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36. Brack C, Lithgow G, Osiewicz H, Toussaint O. EMBO WORKSHOP REPORT: Molecular and cellular gerontology Serpiano, Switzerland, September 18-22, 1999. *EMBO J* 2000; 19:1929-34; PMID:10790359; <http://dx.doi.org/10.1093/emboj/19.9.1929>
37. Chen QM, Bartholomew JC, Campisi J, Acosta M, Reagan JD, Ames BN. Molecular analysis of H<sub>2</sub>O<sub>2</sub>-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. *Biochem J* 1998; 332:43-50; PMID:9576849
38. Fripiat C, Chen QM, Zdanov S, Magalhaes JP, Remacle J, Toussaint O. Subcytotoxic H<sub>2</sub>O<sub>2</sub> stress triggers a release of transforming growth factor-beta 1, which induces biomarkers of cellular senescence of human diploid fibroblasts. *J Biol Chem* 2001; 276:2531-7; PMID:11060295; <http://dx.doi.org/10.1074/jbc.M006809200>
39. Dierick JF, Kalume DE, Wenders F, Salmon M, Dieu M, Raes M, et al. Identification of 30 protein species involved in replicative senescence and stress-induced premature senescence. *FEBS Lett* 2002; 531:499-504; PMID:12435600; [http://dx.doi.org/10.1016/S0014-5793\(02\)03604-9](http://dx.doi.org/10.1016/S0014-5793(02)03604-9)
40. Pascal T, Debacq-Chainiaux F, Chrétien A, Bastin C, Dabée AF, Bertholet V, et al. Comparison of replicative senescence and stress-induced premature senescence combining differential display and low-density DNA arrays. *FEBS Lett* 2005; 579:3651-9; PMID:15963989; <http://dx.doi.org/10.1016/j.febslet.2005.05.056>
41. Sorrell JM, Caplan AI. Fibroblast heterogeneity: more than skin deep. *J Cell Sci* 2004; 117:667-75; PMID:14754903; <http://dx.doi.org/10.1242/jcs.01005>
42. Chainiaux F, Magalhaes JP, Eliaers F, Remacle J, Toussaint O. UVB-induced premature senescence of human diploid skin fibroblasts. *Int J Biochem Cell Biol* 2002; 34:1331-9; PMID:12200029; [http://dx.doi.org/10.1016/S1357-2725\(02\)00022-5](http://dx.doi.org/10.1016/S1357-2725(02)00022-5)
43. Debacq-Chainiaux F, Borlon C, Pascal T, Royer V, Eliaers F, Ninane N, et al. Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. *J Cell Sci* 2005; 118:743-58; PMID:15671065; <http://dx.doi.org/10.1242/jcs.01651>
44. Borlon C, Chretien A, Debacq-Chainiaux F, Toussaint O. Transient increased extracellular release of H<sub>2</sub>O<sub>2</sub> during establishment of UVB-induced premature senescence. *Ann N Y Acad Sci* 2007; 1119:72-7; PMID:18056956; <http://dx.doi.org/10.1196/annals.1404.002>
45. Debacq-Chainiaux F, Borlon C, De Hertogh B, Remacle J, Morvan PY, Vallée R, et al. Identification of potential anti-photoageing algal compounds using an in-vitro model of photoageing. *J Pharm Pharmacol* 2006; 58:1577-83; PMID:17331320; <http://dx.doi.org/10.1211/jpp.58.12.0003>
46. de Magalhães JP, Chainiaux F, Remacle J, Toussaint O. Stress-induced premature senescence in BJ and hTERT-BJ1 human foreskin fibroblasts. *FEBS Lett* 2002; 523:157-62; PMID:12123824; [http://dx.doi.org/10.1016/S0014-5793\(02\)02973-3](http://dx.doi.org/10.1016/S0014-5793(02)02973-3)
47. Massagué J. How cells read TGF-beta signals. *Nat Rev Mol Cell Biol* 2000; 1:169-78; PMID:11252892; <http://dx.doi.org/10.1038/35043051>
48. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci* 2003; 116:217-24; PMID:12482908; <http://dx.doi.org/10.1242/jcs.00229>
49. Debacq-Chainiaux F, Pascal T, Boilan E, Bastin C, Bauwens E, Toussaint O. Screening of senescence-associated genes with specific DNA array reveals the role of IGFBP-3 in premature senescence of human diploid fibroblasts. *Free Radic Biol Med* 2008; 44:1817-32; PMID:18329388; <http://dx.doi.org/10.1016/j.freeradbiomed.2008.02.001>
50. Fripiat C, Dewelle J, Remacle J, Toussaint O. Signal transduction in H<sub>2</sub>O<sub>2</sub>-induced senescence-like phenotype in human diploid fibroblasts. *Free Radic Biol Med* 2002; 33:1334-46; PMID:12419465; [http://dx.doi.org/10.1016/S0891-5849\(02\)01044-4](http://dx.doi.org/10.1016/S0891-5849(02)01044-4)
51. Borlon C, Vankoningsloo S, Godard P, Debacq-Chainiaux F, Toussaint O. Identification of p53-dependent genes potentially involved in UVB-mediated premature senescence of human skin fibroblasts using siRNA technology. *Mech Ageing Dev* 2008; 129:109-19; PMID:18068755; <http://dx.doi.org/10.1016/j.mad.2007.10.014>
52. Herrmann G, Brenneisen P, Wlaschek M, Wenk J, Faiss K, Quel G, et al. Psoralen photoactivation promotes morphological and functional changes in fibroblasts in vitro reminiscent of cellular senescence. *J Cell Sci* 1998; 111:759-67; PMID:9472004
53. Joshi PC, Pathak MA. Production of singlet oxygen and superoxide radicals by psoralens and their biological significance. *Biochem Biophys Res Commun* 1983; 112:638-46; PMID:6303326; [http://dx.doi.org/10.1016/0006-291X\(83\)91511-5](http://dx.doi.org/10.1016/0006-291X(83)91511-5)
54. Ma W, Hommel C, Brenneisen P, Peters T, Smit N, Sedivy J, et al. Long-term growth arrest of PUVA-treated fibroblasts in G2/M in the absence of p16(INK4a) p21(CIP1) or p53. *Exp Dermatol* 2003; 12:629-37; PMID:14705804; <http://dx.doi.org/10.1034/j.1600-0625.2003.00024.x>
55. Borlon C, Debacq-Chainiaux F, Hinrichs C, Scharfetter-Kochanek K, Toussaint O, Wlaschek M. The gene expression profile of psoralen plus UVA-induced premature senescence in skin fibroblasts resembles a combined DNA-damage and stress-induced cellular senescence response phenotype. *Exp Gerontol* 2007; 42:911-23; PMID:17574363; <http://dx.doi.org/10.1016/j.exger.2007.04.009>
56. Ma W, Wlaschek M, Brenneisen P, Schneider LA, Hommel C, Hellweg C, et al. Human dermal fibroblasts escape from the long-term phenocopy of senescence induced by psoralen photoactivation. *Exp Cell Res* 2002; 274:299-309; PMID:11900490; <http://dx.doi.org/10.1006/excr.2002.5476>
57. Ma W, Wlaschek M, Hommel C, Schneider LA, Scharfetter-Kochanek K. Psoralen plus UVA (PUVA) induced premature senescence as a model for stress-induced premature senescence. *Exp Gerontol* 2002; 37:1197-201; PMID:12470831; [http://dx.doi.org/10.1016/S0531-5565\(02\)00143-2](http://dx.doi.org/10.1016/S0531-5565(02)00143-2)
58. Berneburg M, Grether-Beck S, Kürten V, Ruzicka T, Briviba K, Sies H, et al. Singlet oxygen mediates the UVA-induced generation of the photoaging-associated mitochondrial common deletion. *J Biol Chem* 1999; 274:15345-9; PMID:10336420; <http://dx.doi.org/10.1074/jbc.274.22.15345>
59. Eckert RL, Crish JE, Robinson NA. The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. *Physiol Rev* 1997; 77:397-424; PMID:9114819
60. Gosselin K, Deruy E, Martien S, Vercamer C, Bouali F, Dujardin T, et al. Senescent keratinocytes die by autophagic programmed cell death. *Am J Pathol* 2009; 174:423-35; PMID:19147823; <http://dx.doi.org/10.2353/ajpath.2009.080332>
61. Gosselin K, Martien S, Pourtier A, Vercamer C, Ostoich P, Morat L, et al. Senescence-associated oxidative DNA damage promotes the generation of neoplastic cells. *Cancer Res* 2009; 69:7917-25; PMID:19826058; <http://dx.doi.org/10.1158/0008-5472.CAN-08-2510>
62. Soroka Y, Ma'or Z, Leshem Y, Verchovsky L, Neuman R, Brégère FM, et al. Aged keratinocyte phenotyping: morphology, biochemical markers and effects of Dead Sea minerals. *Exp Gerontol* 2008; 43:947-57; PMID:18761079; <http://dx.doi.org/10.1016/j.exger.2008.08.003>
63. Lewis DA, Yi Q, Travers JB, Spandau DF. UVB-induced senescence in human keratinocytes requires a functional insulin-like growth factor-1 receptor and p53. *Mol Biol Cell* 2008; 19:1346-53; PMID:18216278; <http://dx.doi.org/10.1091/mbc.E07-10-1041>
64. Kang MK, Bibb C, Baluda MA, Rey O, Park NH. In vitro replication and differentiation of normal human oral keratinocytes. *Exp Cell Res* 2000; 258:288-97; PMID:10896780; <http://dx.doi.org/10.1006/excr.2000.4943>
65. Kang MK, Kameta A, Shin KH, Baluda MA, Kim HR, Park NH. Senescence-associated genes in normal human oral keratinocytes. *Exp Cell Res* 2003; 287:272-81; PMID:12837283; [http://dx.doi.org/10.1016/S0014-4827\(03\)00061-2](http://dx.doi.org/10.1016/S0014-4827(03)00061-2)
66. Dickson MA, Hahn WC, Ino Y, Ronfard V, Wu JY, Weinberg RA, et al. Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol Cell Biol* 2000; 20:1436-47; PMID:10648628; <http://dx.doi.org/10.1128/MCB.20.4.1436-1447.2000>
67. Bertrand-Vallery V, Boilan E, Ninane N, Demazy C, Friguet B, Toussaint O, et al. Repeated exposures to UVB induce differentiation rather than senescence of human keratinocytes lacking p16(INK4A). *Biogerontology* 2010; 11:167-81; PMID:19554468; <http://dx.doi.org/10.1007/s10522-009-9238-y>
68. Bertrand-Vallery V, Belot N, Dieu M, Delaive E, Ninane N, Demazy C, et al. Proteomic profiling of human keratinocytes undergoing UVB-induced alternative differentiation reveals TRIPartite Motif Protein 29 as a survival factor. *PLoS One* 2010; 5:e10462; PMID:20454669; <http://dx.doi.org/10.1371/journal.pone.0010462>